

**U 013891-8**

**Claims:**

1. A PCR primer set specific for *Leishmania donovani*, said primer set being (1) a first pair of oligonucleotides having the sequences given by SEQ ID NO.1, and SEQ ID NO.2.  
wherein the primer set is effective in a PCR assay for detecting the presence of *Leishmania donovani* infection in samples derived from patients infected by leishmaniasis.
2. A PCR primer set as claimed in claim 1, wherein the primer set is the first pair of oligonucleotides.
3. A PCR primer set as claimed in claim 1 wherein SEQ ID No 1 is 5'-AAATCGGCTCCGAGGCGGGAAAC-3'
4. A PCR primer set as claimed in claim 1 wherein SEQ ID No 2 is 5'-GGTACACTCTATCAGTAGCAC-3'
5. A method of detecting the presence of *Leishmania donovani* in a sample from a patient suspected of leishmaniasis, said method comprising the steps of:
  - a) providing a sample from the patient suspected of being infected with *Leishmania donovani*
  - b) isolating and purifying the nucleic acids from the sample,
  - c) forming a polymerase chain reaction solution containing at least a portion of nucleic acids from step (b), a PCR primer set consisting of SEQ ID Nos. 1 and 2, a mixture of nucleoside triphosphate monomers, and an enzyme *Taq* polymerase in a buffered solution,
  - d) carrying out a polymerase chain reaction on the PCR reaction solution to amplify any *Leishmania donovani*-specific nucleic acid; and
  - e) analysing the *Leishmania donovani*-specific nucleic acids obtained in the polymerase chain reaction using gel-electrophoresis method and staining the resulting gel.

wherein the presence of a band at about 600bp is indicative of the presence of *Leishmania donovani* parasites in the patient.

6) A method as claimed in claim 5 wherein the sample is obtained from peripheral blood or skin lesions of the patient.

7) A method as claimed in claim 5 wherein the nucleic acids are treated with phenol chloroform and ethanol to isolate/purify them.

8) A method as claimed in claim 5 wherein the primers are sensitive so as to detect even 10 fg *Leishmania* DNA diluted in 10 million fold excess of human DNA in PCR reactions.

9) A method as claimed in claim 5 wherein the PCR reaction is performed in a thermal cycler overlaid with mineral oil.

10) A PCR primer set as claimed in claim 1 wherein SEQ ID No 1 is 5'-AAATCGGCTCCGAGGCGGGAAAC-3'

11) A PCR primer set as claimed in claim 1 wherein SEQ ID No 2 is 5'-GGTACACTCTATCAGTAGCAC-3'

12) A method as claimed in claim 5 wherein steps of amplifying the *Leishmania donovani*-specific nucleic acid comprises initial denaturation at 94°C for 2 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 2 min, and a final extension at 72°C is carried out for 3 min so that multiple copies of the *Leishmania donovani* specific nucleic acid are produced.

13) A kit for detecting *Leishmania donovani* in a sample, comprising oligonucleotide primers, wherein the primers comprise SEQ ID No 1 and SEQ ID No 2, and wherein the primers specifically hybridize to the said *Leishmania donovani*.

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